Original Article
Superiority of sucrase-isomaltase to CD10 for immunohistochemical detection of intestinal absorptive cell phenotype in differentiated-type gastric adenocarcinoma

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Abstract: Gastric adenocarcinoma (GAC) can be divided immunophenotypically into gastric, intestinal, or mixed gastric and intestinal phenotypes. Cadherin 17 (CDH17) and CD10 have been used as comprehensive markers for intestinal epithelial cells and for small intestinal absorptive cells in GACs, respectively. Sucrase-isomaltase (SI) and CD10 are expressed in small intestinal absorptive cells and SI is more frequently expressed than CD10 in gastric intestinal metaplasia (IM). The aim of this study was to evaluate the potential of SI as a marker for intestinal absorptive cells compared to CD10 in differentiated-type (DT) GACs. We compared the immunohistochemical expression of CDH17, SI, and CD10 in IMs and tissue microarrays of 40 samples of DTGACs. In IMs and DTGACs, CDH17 showed a diffuse lateral cytoplasmic membrane staining both in columnar and goblet cells. SI and CD10 were expressed on the luminal surfaces of the columnar cells. In IMs, SI was positive both in both complete-type IMs and in incomplete-type IMs. CD10 was positive only in complete-type IMs. In DTGACs, CDH17, SI, and CD 10 were positive in 37 (92.5%), 22 (55%), and 11 (27.5%) cases, respectively. In SI-positive cases, the degrees of expression of SI were equal to (7 cases) or less than (15 cases) those of CDH17; the degrees of expression of SI were equal to (5 cases), more than (16 cases), or less than (1 case) those of CD10. In conclusion, SI is a more sensitive immunohistochemical marker for intestinal absorptive cells than CD10 in DTGACs.

Keywords: Gastric cancer, CD10, immunohistochemistry, intestinal metaplasia, sucrase-isomaltase

Introduction

Gastric adenocarcinoma (GAC) can be divided immunophenotypically into gastric, intestinal, or mixed gastric and intestinal phenotypes. This immunophenotypic classification has provided useful information for understanding gastric cancer pathogenesis: gastric and intestinal phenotypes of differentiated-type (DT) GAC (DTGAC) have distinct clinical, genetic, and epigenetic characteristics [1].

CD10 has been used as an immunophenotypic marker for small intestinal absorptive cells in DTGACs [2, 3]. CD10 is a cell membrane-bound neutral endopeptidase and expressed normally in the brush border of absorptive cells of the small intestine [4]. CD10 is expressed in metaplastic absorptive cells in complete-type intestinal metaplasia (IM), but not in incomplete-type IM [5], in which metaplastic absorptive cells have shorter and more sparsely distributed microvilli than those in complete-type IM [6] and seem to be immature, suggesting immunostaining for CD10 may underestimate absorptive cell-phenotype in GACs.

Sucrase-isomaltase (SI), which is normally located on the brush border of absorptive cells of the small intestine, was demonstrated in both complete and in incomplete-type gastric IM using enzyme histochemical techniques [7],

and also demonstrated immunohistochemically in DTGACs [8, 9].

As a comprehensive cell lineage marker in assessing the intestinal phenotype of GACs, cadherin 17 (CDH17) is useful [10-12]. CDH17 is a cell-adhesion protein expressed in intestinal goblet cells and enterocytes in the human gastrointestinal tract [13]. In gastrointestinal cancers, immunostaining for CDH17 exhibited higher sensitivity than CK20 and CDX2 and showed similar specificity to CDX2 [13, 14].

In this study, therefore, we sought to reevaluate the potential of SI as an immunophenotypic marker for absorptive cells, in comparison with CDH17 and CD10 in DTGACs.

Materials and methods

Tissue samples

A total of 40 cases of DTGACs were retrieved from the pathology archive of the department of laboratory medicine, Shinshu University Hospital, Matsumoto, Nagano, Japan. From each case, a representative block with a tumor was collected. Only cases with sufficient tumor tissue in the paraffin block were selected. All specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. The most representative region of differentiated-type adenocarcinoma was selected based on the hematoxylin and eosin (H&E)-stained slide. From each corresponding paraffin block, tissue cores were punched out using thin-walled 5 × 5 mm stainless steel needles (Azumaya Medical Instruments Inc., Tokyo, Japan), and cores were arrayed in paraffin blocks. Additionally, normal gastric mucosa, gastric mucosa with intestinal metaplasia, and normal duodenal mucosa were also obtained from four gastrectomy specimens with proximal duodenum resected for GAC. Serial sections of 3 μm thickness were cut from these blocks, stained with H&E for histological examination, and subjected to immunohistochemistry.

Immunohistochemistry

Immunohistochemistry was performed using three different antibodies: CDH17 (clone SP183, 1:200; Cell Marque, Rocklin, CA, USA); SI (PAD186Hu01, 1:1000; Cloud-Clone Corp, Katy, TX, USA), and CD10 (clone 556C6, 1:200;

Leica Biosystems, Wetzlar, Germany). Paraffin sections were deparaffinized, rehydrated, and placed in a 0.3% hydrogen peroxide solution in methanol for 30 min at room temperature to block endogenous peroxide activity. Antigen retrieval was performed using the following methods: samples were heated in Histofine antigen retrieval solution (pH 9.0, Nichirei Biosciences, Tokyo, Japan for CDH17, and CD10; pH 6.0, LSI Medience, Tokyo, Japan for SI) using a Decloaking Chamber NxGen (Biocare Medical, Pacheco, CA, USA) at 110°C for 10 min.

Slides were incubated with the primary antibodies for 2 h at room temperature. Subsequently, they were incubated with Novocastra Novolink™ (Leica Biosystems) for CDH17 and Histofine Simple Stain MAX PO Multi (Nichirei Biosciences) for SI and CD10. The sections were visualized using a DAB substrate solution. Finally, the slides were counterstained with hematoxylin, dehydrated, and mounted. Normal duodenal mucosa served as positive control for CDH17, SI, and CD10.

Evaluation of immunostaining

The approximate proportion of positively immunostained cells in the lesions was evaluated based on a five-grade semiquantitative scale as follows: score 0 (0-4%), 1 (5-24%), 2 (25-49%), 3 (50-74%), and 4 (75% and more). A lesion was considered positive when at least 5% of the tumor cells displayed immunohistochemical staining, irrespective of the intensity. Staining scores are non-parametric and are thus expressed as a median score with the interquartile range. All samples were reviewed independently by two observers (H.O. and T.U.). To resolve intra-observer variation, all specimens were assessed on two separate occasions. If the scores differed between the different investigators, the slides were reevaluated on a multi-head microscope until consensus was achieved.

This study was approved by the ethics committee of Shinshu University (approval number 3749).

Statistical analysis

The Mann-Whitney U test was used to compare the staining scores for immunophenotypic
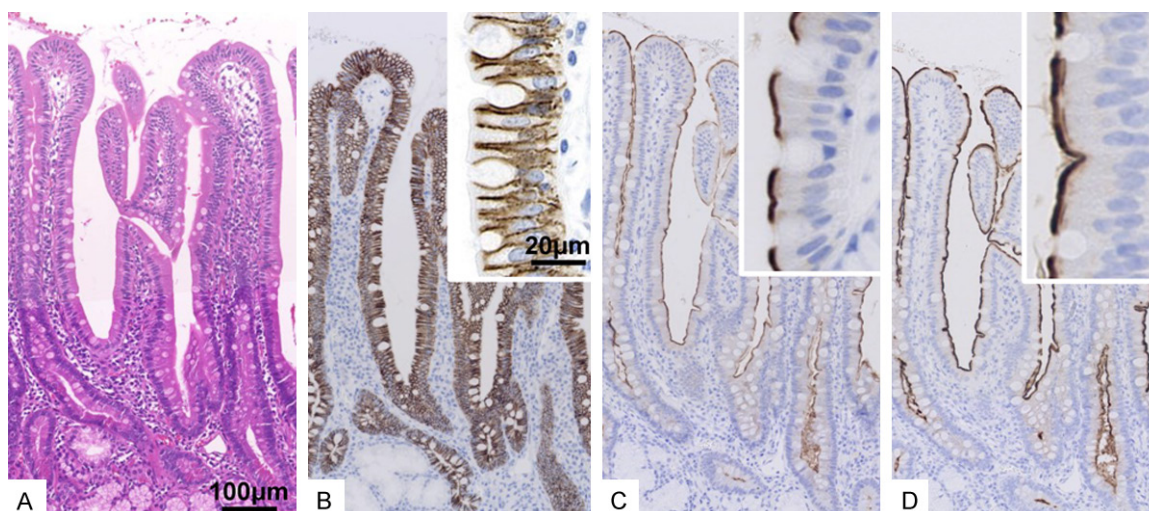
Figure 1. Normal duodenal mucosa. All figures were prepared from serial sections. (A) Hematoxylin and eosin staining, (B) cadherin 17 shows a diffuse lateral cytoplasmic membrane staining in intestinal cells, (C) expression of sucrase-isomaltase is evident on the luminal surface of the absorptive cells, and (D) expression of CD10 is evident on the luminal surface of absorptive cells. (60× magnification, 300× magnification [inset]).

markers. $P < 0.05$ was considered significant. All statistical analyses were performed using Bell Curve for Excel software (Social Survey Research Information, Tokoyo, Japan).

Results

Normal gastric mucosa

CDH17, SI and CD10 were negative in normal gastric epithelial cells.

Normal duodenal mucosa

CDH17 (**Figure 1A** and **1B**) showed a diffuse lateral cytoplasmic membrane staining both in absorptive cells and goblet cells. SI (**Figure 1A** and **1C**) and CD10 (**Figure 1A** and **1D**) showed strong positivity of the luminal surface of absorptive cells.

Intestinal metaplasia

In both complete and in incomplete-type IM, CDH17 (**Figures 2A**, **2B**, **3A** and **3B**) showed reactivity similar to that of normal duodenal mucosa: diffuse lateral cytoplasmic membrane staining both in columnar cells and goblet cells. SI and CD10 demonstrated strong staining positivity on the luminal surface of the columnar cells in complete-type IM (**Figure 2A**, **2C** and **2D**); however, columnar cells in incomplete-type IM were positive only for SI (**Figure 3A** and **3C**) and negative for CD10 (**Figure 3A** and **3D**).

Adenocarcinoma

CDH17 (**Figure 4A** and **4B**) exhibited a lateral cytoplasmic membrane staining in the carcinoma cells. Expression of SI (**Figure 4A** and **4C**) and CD10 (**Figure 4A** and **4D**) was evident on the luminal surfaces of the carcinoma cells in the glandular pattern.

CDH17, SI and CD 10 were positive in 37 (92.5%), 22 (55%), and 11 (27.5%) cases, respectively. The staining score for SI (median [interquartile range]) (1 [0, 2]) was significantly higher than that of CD10 (0 [0, 1]) ($P < 0.01$, **Figure 5**). The degrees of expression of intestinal markers, in decreasing order of scores, were of CDH17 (median [interquartile range]) (3 [1, 4]), SI (1 [0, 2]), and CD10 (0 [0, 1]) ($P < 0.01$) (**Figure 5**). In SI-positive cases, the degrees of expression of SI were equal to (7 cases) or less than (15 cases) those of CDH17; the degrees of expression of SI were equal to (5 cases), more than (16 cases), or less than (1 case) those of CD10.

Discussion

The present study compared immunohistochemical expression of CDH17, SI, and CD10 in gastric IMs and DTGACs. SI was more frequently expressed than CD10 in gastric IMs and in DTGACs, and the degrees of expression of intestinal markers, in decreasing order, were CDH17, SI, and CD10 ($P < 0.01$). In gastric IMs,
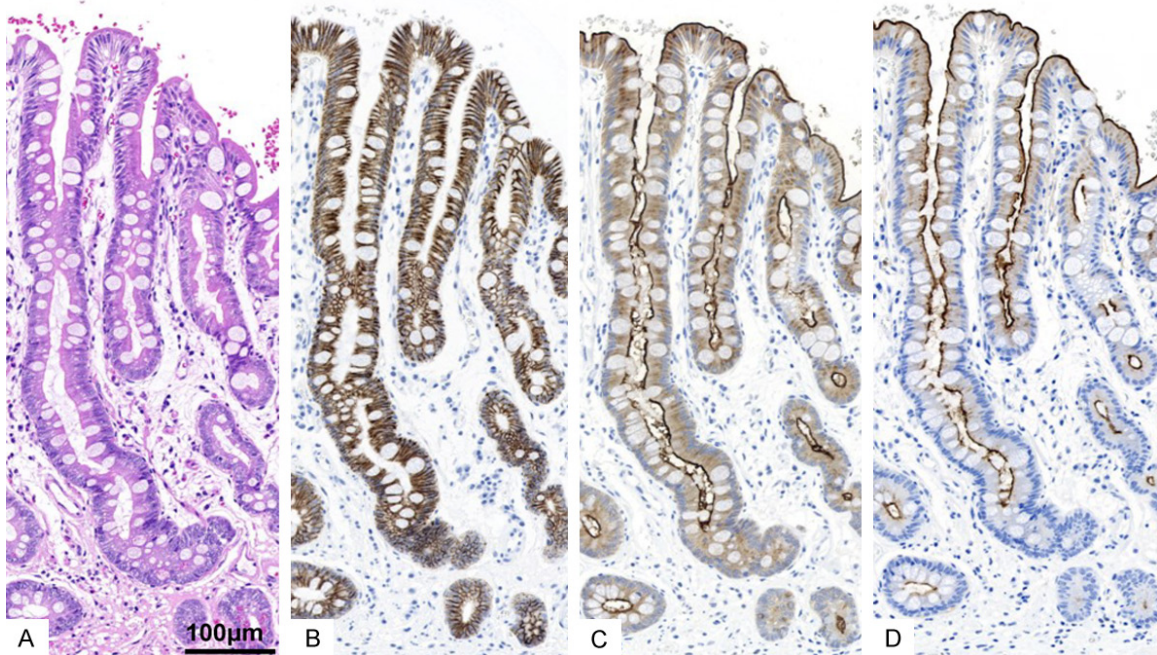
Figure 2. Gastric intestinal metaplasia, complete type. All figures were prepared from serial sections. (A) Hematoxylin and eosin staining, (B) cadherin 17 shows a diffuse lateral cytoplasmic membrane staining in metaplastic cells, (C) expression of sucrase-isomaltase is evident on the luminal surface of metaplastic cells, and (D) expression of CD10 is evident on the luminal surface of metaplastic cells. (100× magnification).
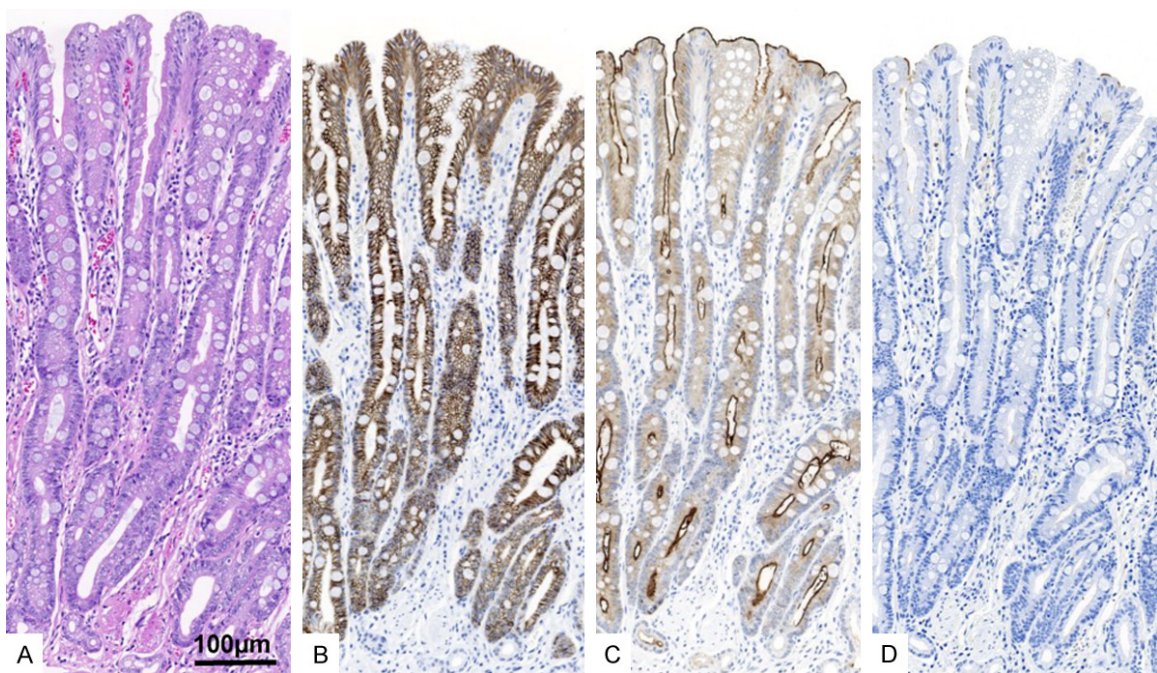

Figure 3. Gastric intestinal metaplasia, incomplete type. All figures were prepared from serial sections. (A) Hematoxylin and eosin staining, (B) cadherin 17 shows a diffuse lateral cytoplasmic membrane staining in metaplastic cells, (C) expression of sucrase-isomaltase is evident on the luminal surface of metaplastic cells, and (D) CD10 is negative in metaplastic cells. (90× magnification).

CDH17 showed diffuse lateral cytoplasmic membrane staining both in columnar cells and goblet cells, and SI showed strong staining positivity on the luminal surface of the columnar
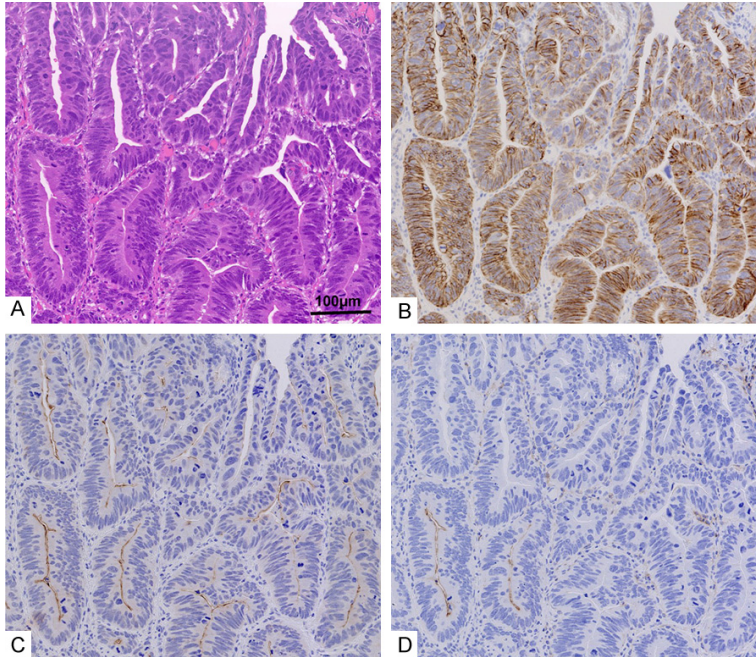
Figure 4. Well-differentiated tubular adenocarcinoma. All figures were prepared from serial sections. (A) Hematoxylin and eosin staining, (B) cadherin 17 shows a diffuse lateral cytoplasmic membrane staining in carcinoma cells, (C) sucrase-isomaltase is diffusely expressed on the luminal surface of carcinoma cells, and (D) CD10 is focally expressed on the luminal surface of carcinoma cells. (120× magnification).

in DTGACs has been obtained in other studies. Ito et al. [10] reported CDH17 expression in 74% of DTGACs. Park et al. [16] observed CDH17 expression in 83% in well and moderately differentiated GACs.

In this study, SI was positive in 55% of DTGACs. Our result is consistent with that reported by Nakamura et al. [8], in which 51.9% of DTGACs were positive for SI. SI is expressed also in the apical region of normal colonic crypt cells as well as in the brush border of absorptive cells of the small intestine, although colonic SI is a distinct form with relatively low levels of enzyme activities in contrast to that of the small intestinal SI [17]. However, immunohistochemical expression of SI in DTGAC may reflect the small intestinal absorptive cell phenotype because the colonic phenotype occurs rarely in gastric carcinogenesis [9, 18].
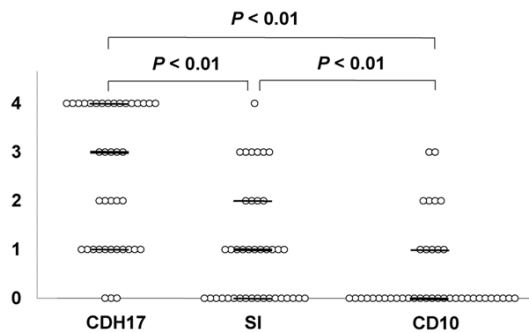

Figure 5. Staining scores for cadherin 17 (CDH17), sucrase-isomaltase (SI), and CD10 in differentiated type gastric adenocarcinomas. The staining score for CDH17 was significantly higher than that for SI and CD10 ($P < 0.01$). The staining score for SI was significantly higher than that for CD10 ($P < 0.01$). Scores for staining were analyzed by the Mann-Whitney U-test.

cells, similar to results obtained by Grotzinger et al. [15] where expression of CDH17 was observed in all cases of gastric IMs confirmed by MUC2-positive goblet cells; whereas SI failed to detect gastric IMs in some of those cases.

CDH17 positivity in this study was 92.5% in DTGACs. A similar high positive rate of CDH17

This study demonstrated CD10 positivity in 27.5% of DTGACs. Similar results were obtained from other studies. Tajima et al. reported CD10 expression in 22.7% of early DTGACs [3] and 36.2% of advanced DTGACs [2]. Shiroshita et al. [19] also reported that CD10 was positive in 24.2% of early DTGACs, in 28.2% of advanced DTGACs, and 25.1% of early and advanced DTGACs.

We found that SI exhibited higher sensitivity than CD10 in identifying intestinal absorptive phenotype in gastric IMs and DTGACs. As confirmed in this study, in gastric IM, SI was demonstrated both in complete and in incomplete-type IM using enzyme histochemical techniques [7], and immunohistochemical expression of CD10 was found in complete-type IM [5], but not in incomplete-type IM [5]. Considering that absorptive cells in gastric IMs appear to be less differentiated in incomplete-type IM than in complete type-IM based on ultrastructure [6] and that SI is expressed very early during fetal development, by 10 weeks of

gestation [20], it is suggested that SI is more frequently expressed in immature absorptive cells than CD10 in gastric IMs and DTGACs. Alternatively, immunostaining for CD10 is likely to underestimate the phenotypic expression of the intestinal absorptive cells compared to that for SI in gastric IMs and DTGACs.

In conclusion, for the estimation of intestinal phenotype in DTGACs, SI is a more sensitive immunohistochemical marker for absorptive cells than CD10.

Acknowledgements

Disclosure of conflict of interest

None.

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